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PROGRESS REPORT

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

1 July through 30 September 1965

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PREPARED UNDER CONTRACT NASw-812

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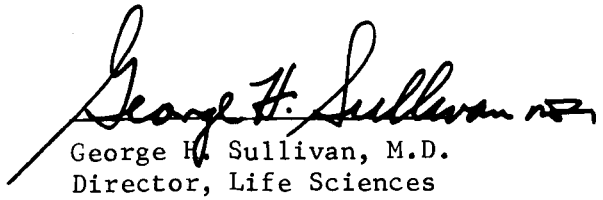

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INTESTINAL MUCOSA DYNAMICS OF THE LITTLE POCKET MOUSE (PEROGNATHUS LONGIMEMBRIS)

J. J. Gambino and J. W. Towner

Introduction

Earlier we suggested that pocket mouse radiation resistance might be due, in part, to a slow rate of loss of epithelial cells from the intestinal villi (3). One consequence of a slow sloughing rate would be a slower rate of villus attrition and, effectively, protection from the gastrointestinal syndrome. This suggestion was predicated on the fact that no deaths occur in pocket mice during the first week after high dose whole body irradiation, a time when conventional mice and rats succumb to the gastrointestinal syndrome. Furthermore, dose survival time curves of pocket mice and germfree mice show remarkable similarities, with the plateau representing gastrointestinal death displaced upward and shortened for both species (1,5,6). Since this shift of the plateau was attributed to certain peculiarities in cell population dynamics of the intestinal mucosa of germfree mice, similar mechanisms were sought in pocket mice.

In two experiments that are reported herein, early post-irradiation histopathology of the intestine was examined, and the normal transit-time of pocket mouse intestinal epithelial cells was determined.

Methods and Materials

Field trapped, adult Perognathus longimembris, which had been maintained in our laboratory for approximately one year were used. Environmental conditions and animal husbandry are described elsewhere (2,3). Ambient temperature and humidity were maintained at 22° $\pm$ 2°C and 50 $\pm$ 5%, respectively. The animals were individually housed and fed a mixture of grass seeds and sunflower seeds. Since pocket mice have no requirement for drinking water, no water was provided. Lights were turned on at 0630 hours and off at 0530 hours PST.

Twenty-five male pocket mice were used in the first experiment. They were divided into 3 groups: two groups were given a single, acute whole body dose of either 1000 rad or 1500 rad, and one was kept as control.

Irradiation was delivered from a 5000 curie Co^{60} source at 24.5 rad/min for the 1000 rad dose, and at 37.8 rad/min for the 1500 rad dose. Ferric sulphate and phosphate glass dosimetry was used.

Animals were sacrificed at selected intervals up to one week post-irradiation. Sections of small intestine were removed and fixed in 10% formalin, sectioned at 7μ and stained with haematoxylin-eosin.

For the second experiment 20 P. longimembris of both sexes were randomly divided into 5 groups of 4 mice each. All 20 animals were administered 10μc of tritiated thymidine (Schwarz Bioresearch, Inc. Lot #1402, Sp. Ac. 0.36c/mM) in 0.1 ml physiological saline intraperitoneally at the start of the experiment.

At 12, 24, 72, 120 and 168 hours post-injection, four animals were sacrificed by ether anaesthesia and a section of jejunum was fixed in Bouin's.

Autoradiographs were prepared in the usual manner with Kodak NTB-2 nuclear track emulsion*. Some were developed after 10 days and some after 30 days exposure. Haematoxylin-eosin stain was used after development.

Two slides were prepared for each animal, providing ample material for scanning. The percentage of villus height traversed by labeled cells was judged by scanning all the sections prepared for each animal and measuring the distance traveled by the most advanced labeled cell front. This "front" appeared to be sufficiently representative of all the labeled cells on any particular slide.

Results

The intestinal mucosa of pocket mice sacrificed at 6 and 15 hours after receiving 1000 rad and 1500 rad total body gamma irradiation showed the usual degenerative changes of irradiation damage. Villi remained intact but the villus epithelial cells showed increased cytoplasmic vacuolation and some distortion in size and shape. Nuclei of villus epithelial cells remained relatively uniform in size and staining qualities. Cells of the crypts of Lieberkühn showed much greater changes than those of the villi. Their cytoplasm was highly vacuolated and considerable nuclear damage was evident. Chromatin debris and cellular fragments predominated in the crypts. Few mitotic figures could be seen, and those that were visible appeared abnormal.

*Appreciation is expressed to Dr. A. C. Upton and William D. Gude of Oak Ridge National Laboratory for the preparation of these autoradiographs.

At 30 to 39 hours post-irradiation the villi were still intact, although they appeared to be slightly shortened. Nuclear and other cellular debris was cleared from most of the crypts, and a few mitotic figures were observed.

By the 4th day post-irradiation, crypts appeared normal except for an increased rate of mitosis as compared with controls.

Between the 5th and 7th days, movement of regenerated epithelial cells up the villus was well marked by the characteristic basophilic staining of new cells. It was at this time that differences in the 1000 rad and 1500 rad groups were seen. By day 6 the replacement of villus epithelium was completed in the 1000 rad animals, while the 1500 rad animals showed the regeneration wave only part way up the villus. It was difficult to differentiate between control slides and slides of either of the irradiated groups on day 7.

During the course of the post-irradiation period, neither irradiated group showed appreciable degeneration of the villus. At these dose levels villus epithelial cells tended to remain intact on the villus core until replaced by regenerated cells.

Figure 1 shows the percentage of villus height traversed by labeled cells at intervals in the 7 days following injection with tritiated thymidine.

Although the data were not obtained in precisely the same manner, Figure 2 compares transit times of pocket mice, germfree mice and conventional mice (5). The points plotted in Figure 2 for the pocket mouse were obtained by averaging the distances represented by bars in Figure 1. These data demonstrate a longer villus transit time for pocket mouse epithelial cells, than for either germfree or conventional mice. The estimated times for reaching the villus tip are 2.1 days for conventional mice, 4.3 days for germfree mice, and 5.7 for pocket mice.

Discussion

Damage to the gastrointestinal mucosa is responsible in a large measure for early deaths in mammals after whole body radiation doses ranging between 1000 and 10,000 rad. In this dose range, death is generally attributed to denudation of the intestinal mucosa with concomitant bloody diarrhea, electrolyte loss, bacterial invasion of tissues, etc., which are all manifestations of the acute gastrointestinal syndrome. Germfree mice and

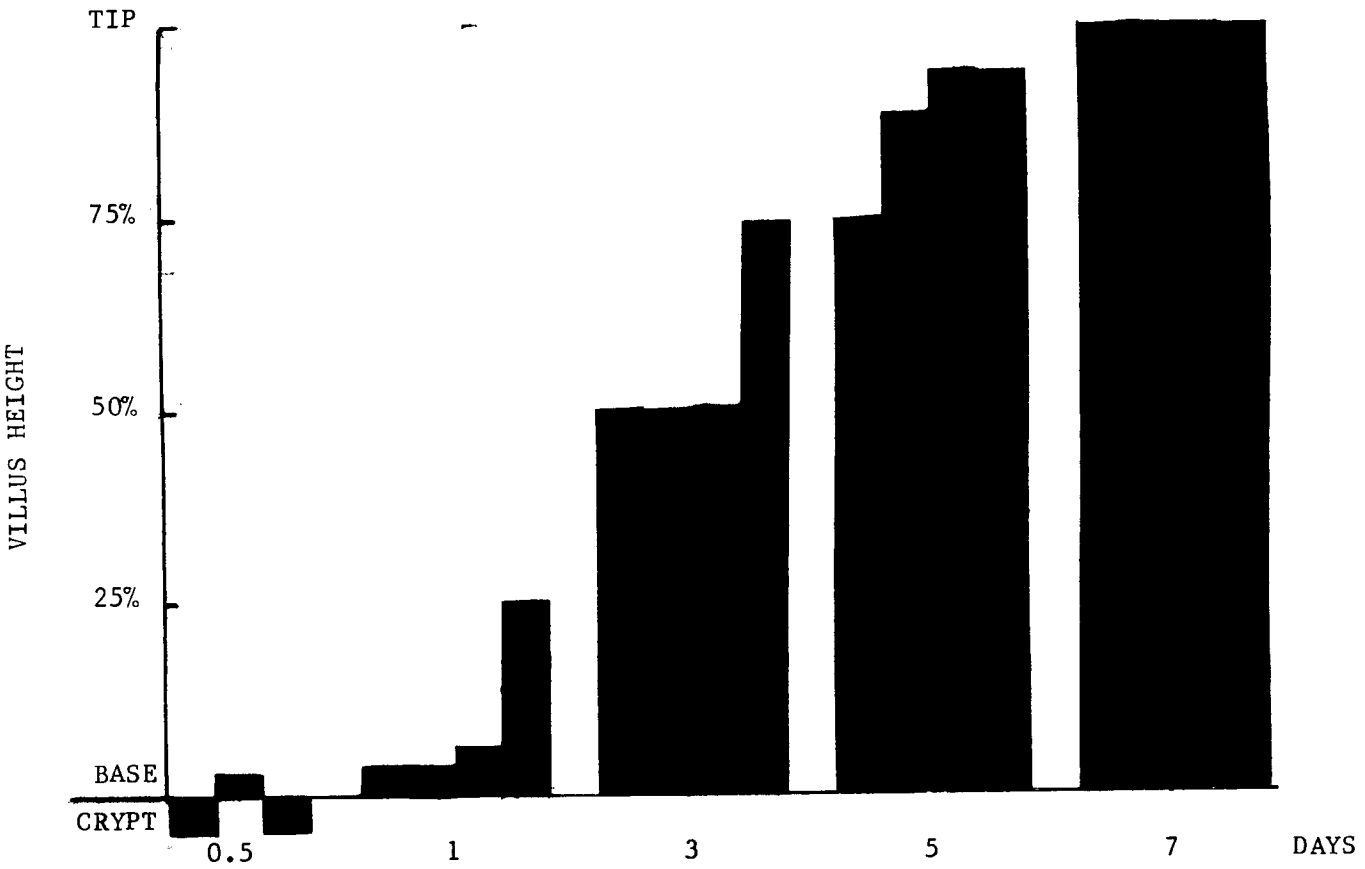

FIGURE 1 - Progress of thymidine-H^3-labeled cells on villi of pocket mouse jejunum expressed as percentage of villus height traversed by the labeled cell "front". Each bar represents one animal.

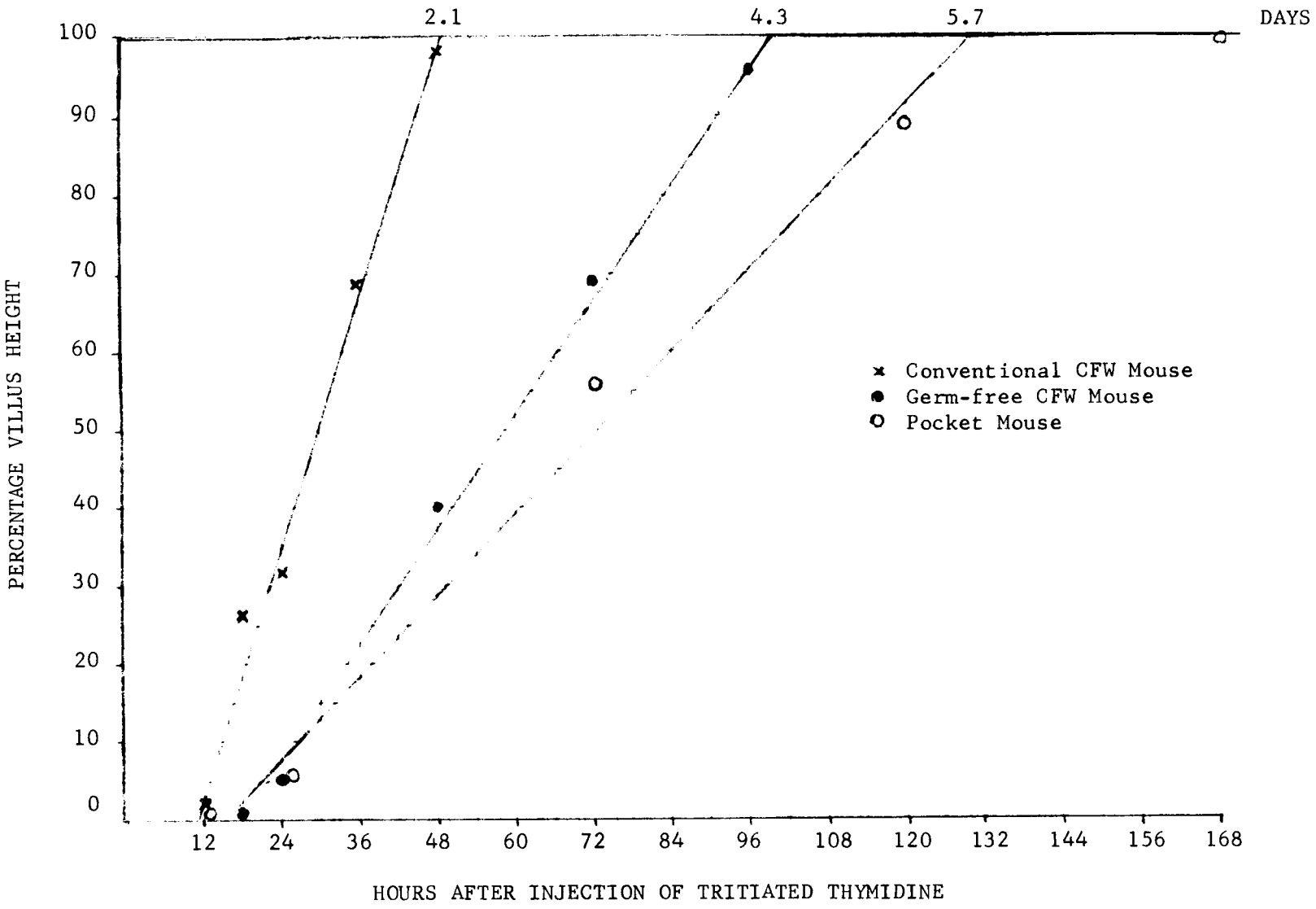

FIGURE 2 - Progress of thymidine-H^3-labeled cells on villi of pocket mice compared with values for germfree and conventional mice reported by Matsuzawa and Wilson (Rad. Res. 25, 15-24, 1965).

several species of wild rodents, which show various degrees of radiation resistance, appear to bypass the acute gastrointestinal syndrome (2,4,5,6). It has been suggested for germfree mice that survival is the result of a much slower rate of villus cell loss. This slow loss of epithelial cells tends to maintain the villi intact after high dose irradiation, thereby enhancing survival. For example, conventional mice and rats, if given supralethal doses, die at about 2 to 4 days post-irradiation. The 2 to 4 day time period is about twice as long as villus transit time, which in turn is a reliable measure of the rate of cell loss at the villus tip. Germfree mice, on the other hand, have a longer survival time after equivalent doses, generally surviving for 7 days (5,6). This survival time, too, is approximately double the villus transit time for germfree mice.

Since germfree mice have a slower rate of sloughing at the villus tip than do conventional mice, the entire cell renewal system involved in replenishing the gut mucosa is slowed. Therefore, other factors, such as decreased mitotic activity, and decreased cellular activity, as well as the absence of bacteria all operate to produce the net effect in germfree mice.

Pocket mice, which survive 8 to 12 days following supralethal irradiation, have now been shown to have an even longer transit time than germfree mice. Since transit time is considered a measure of sloughing rate, much of the radiation resistance of pocket mice can be attributed to the fact that the integrity of the gastrointestinal epithelium is maintained even after high dose irradiation. The histological picture of pocket mouse intestine after 1500 rad irradiation tends to corroborate this point.

Summary

Pocket mice subjected to 1000 rad and 1500 rad whole body Co^{60} irradiation were sacrificed in the one week period following exposure. Histopathology of the intestinal mucosa was examined. Degenerative changes were noted within hours after irradiation, but damage was confined mainly to intestinal crypts, while villi remained intact throughout the period of observation. Regeneration of epithelial cells was prompt, and even at the 1500 rad dose level, was completed by about 7 days post-irradiation.

Tritiated thymidine studies indicate that pocket mice have a villus transit time of 5.7 days for intestinal mucosa cells in contrast to 4.3 days and 2.1 days for germfree and conventional CFW mice, respectively. The differences between germfree and conventional CFW mice and the marked contrast between CFW mice and pocket mice in response to a given dose of radiation may reflect, in part, these differences in villus transit time.

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GROWTH AND DEVELOPMENT OF PEROGNATHUS LONGIMEMBRIS

P. Hayden and J. J. Gambino

Introduction

Growth and development data for the Heteromyid rodents are very limited. This group is composed of kangaroo rats, pocket mice and kangaroo mice, nearly all of which are noted for their adaptation to an arid environment. These animals are solitary in nature and exhibit strong territorial behavior. Because of this, most attempts to breed them in captivity have been singularly unsuccessful. However, several species of the genus Dipodomys have been bred in the laboratory, and various details of mating behavior, growth and development are available (Day et al, 1956; Chew, 1958; Chew and Butterworth, 1959; Butterworth, 1961a,b).

More recently, several species of pocket mice (genus Perognathus) have been mated under laboratory conditions (Eisenberg and Isaac, 1963). However, only limited growth data is presented on 3 litters of P. californicus, one litter of P. penicillatus and one litter of P. flavus. Successful breeding of P. longimembris and preliminary data on growth and development were reported earlier from this laboratory (Hayden et al, 1965). The current report presents representative data from 61 litters of P. longimembris that have been bred in our laboratory between April and September, 1965.

Methods and Materials

Details of growth and development were obtained for eight litters with a total of 26 individuals. One of the litters was field-conceived, while the rest were laboratory-bred. All of the females were from the Mojave Desert of California (Whitewater Canyon area, about 10 miles east of Palm Springs). The data group was reduced to 24 animals at 14 days and to 22 at 32 days because of death or escape of individuals.

Females and their litters were housed in galvanized boxes, 8" x 11" x 6.5" with screen wire tops. Sand approximately 1-2 inches deep was provided in the cage for digging and grooming. A container (pint can, milk carton, etc.) was provided for nesting. Dry grass (timothy and rye or bermuda)

was placed in the container for nest material. Torn paper towels were used in addition to the grass in several cases. Obviously gravid females were placed in the maternity cages 2-8 days prior to expected parturition date to acclimate the female to the new environment. If the female appeared nervous or easily agitated, the screen top was covered with a towel, and her cage placed in a more isolated position in the animal holding facility.

The diet was provided ad libitum and consisted of about equal parts of hulled sunflower seed, rye grain, oat groats, hulled millet, parakeet mix, rye grass seed and rye grass seed enriched with water soluble vitamins (Avitron, trade name). Pieces of raw carrots were provided every other day.

Litters were handled only after the hands were washed thoroughly with soap and water, rinsed in ethyl alcohol and dried. The first few litters were handled hesitantly, but it was found that they could be manipulated from the first day of life with no apparent adverse effects on the neonates or on the adequacy of maternal care.

Measurements were taken routinely on specific days of the week. However, since litters were added to the sample group as they were born, animals in the various litters were not all measured at precisely the same age. For this reason, plotted values do not represent the same number of individuals. This fact was taken into consideration by subjectively weighting the points when the curves were visually fitted. The following measurements were made on all animals: body weight, total body length, tail length, hind foot length and ear length. Tail and body measurements were taken with a flexible plastic millimeter ruler, while hind foot and ear were taken with vernier calipers. Linear measurements were read to 0.5 mm (except ear, to .1 mm), weights to 0.01 gm.

Measurements of total body length were taken on active juveniles while the animal, held by the tail, extended itself in an escape attempt. Tail measurements were taken from the same position, using slight pressure of the ruler against the base of the tail. Linear measurements on live animals are subject to inherent errors and recorded values should be treated accordingly.

Measurement data were analyzed as in Brody (1945). Measurement values were plotted on a logarithmic scale versus age on an arithmetic scale. Linear segments of such a plot indicate periods when growth increments were a constant percentage of previous size. From these linear sections, instantaneous growth rates were calculated as:

$$k = \frac{\ln m_2 - \ln m_1}{t_2 - t_1}$$

The value k is the instantaneous percentage rate of growth for the unit of time in which t_2 and t_1 are expressed; $\ln m_2$ and $\ln m_1$ are natural logarithms of the measurements made at t_1 and t_2.

General Development

At birth, the skin is hairless, pink, wrinkled and nearly transparent. The internal organs can be clearly seen through the ventral surface, as can blood vessels, brain and sutures in the skull. Vibrissae are present on the snout at birth and are about 1-1.5 mm long.

Dark pigmentation starts to appear on the head and back at 4-6 days and eventually covers the body down to the lateral line area of the adult. Sparse dark gray hair appears on the pigmented area of the dorsum at 7-9 days with rather coarse white hair on the flanks and legs. By day 13-15 all the animals are covered with a full coat of juvenile pelage, gray on the back, whitish underside and with a buff cast to the head region. The adult colored pelage of pinkish or ochraceous-buff overlaid with blackish hairs on the dorsal surface and ventral surface of pale tawny to buffy white starts to appear between 29-40 days. Color changes seem to appear first on the back of the head or under the eyes.

The ears, sealed at birth, appear as protuberances. At 3-4 days, a groove deepens on the anterior surface of the auditory protuberance and forms the pinnae. By day 5, all pinnae were unfolded and are about 1 mm long. The meatus still appeared to be closed at 13 days and exact time of opening was not determined.

The eyes are sealed at birth and appear as large, heavily pigmented areas behind the thin integument. Eyelids start to develop at 6-7 days and are well formed by day 14. In most of the litters, the eyes were open by day 14-15, but one litter did not open until day 18. These data agree

with those presented for P. californicus, penicillatus and flavus (Eisenberg and Isaac, 1963).

The toenails are not evident at birth but are quite distinct by day 3. The incisors penetrate the gums as early as day 5 and are evident in all young by day 11. Cheek-pouches are not visible on the newborn. By day 3, a crease marking the future fur-lined pouch of adult is noted. This crease gradually invaginates and by day 10 is approximately 1/8" deep. The pouch increases in depth and is lightly haired by the time the juvenile pelage is acquired. The external genitalia are very similar in both sexes but can be fairly well distinguished by 14 days. One female was noted to become sexually active at 60 days (vulva open and swollen). No males have been noted to have descended testes up to 150 days (April-September).

Behavior

All neonates exhibit the righting reflex and were able to crawl, although very laboriously. Ability to move increased daily and by day 6, one animal was noted to perform the characteristic digging motion involving coordinated movement of fore and hind legs. This occurred when the animal was placed upon a metal pan in preparation for weighing and was obviously disturbed by the cold metal. By day 8, the young moved about the cage freely and by day 12 were noted to be gamboling about, even though their eyes were still sealed.

Young mice have been noted to eat raw carrots at 10 days and seeds have been found in pouches by day 14. It was not determined at what age the animals are naturally weaned, but they appear to become self-sufficient at about 18 days. One litter survived when the mother died at 14 days.

Siblings were normally separated and placed in individual containers at about 30 days. On occasion, they were separated as early as 21 days or as late as 42 days. Sibling aggression and resultant injury or death was noted in several litters less than 30 days old.

When neonates were carried by the dam, they drew their legs up close to their body, thereby presenting the minimal number of protuberances to impede progress. This same reflex was noted when the young animals were picked up by the loose skin of the back during measurements.

Weight and Measurements

When weight values are plotted on a logarithmic scale, there are several phases of growth during which the percentage of increase per day is constant. These values of weight increase are given in Fig. 1. There are apparently four distinct growth phases. The first phase of almost 7.4% growth per day extends from day 0 to 21. This represents a linear increase to 65% of adult weight in 21 days. Comparative data for P. californicus (Eisenberg and Isaac, 1963) shows this species attaining only 39% of adult weight during the same time. Unfortunately, comparative data for P. flavus, which is similar in size to P. longimembris, is not available, although this species has been bred in captivity (one litter, Eisenberg and Isaac, 1963). The growth rates of other heteromyid rodents (Chew and Butterworth, 1959; Butterworth, 1961) indicate that the initial increase is greater, although it is not sustained as long as P. longimembris. According to Chew and Butterworth, kangaroo rats attain about 30-50% of maximum weight during this first 20-day period.

Growth rates for P. longimembris level off by about 50-60 days, at which time near maximum weight has been attained. After that time, body weight appears to increase at a rate of about 0.02% per day. Maximum body weight probably is not a meaningful value, because it is a point that fluctuates seasonally as well as daily.

Inflections in weight increase are suggested to be associated with other milestones in the development of wild rodents. Chew and Butterworth (1963) note that the first inflection of the curve for the kangaroo rat, D. merriami, coincides with opening of the eyes and ears. This was not observed in P. longimembris. Spontaneous weaning or self-sufficiency was estimated to occur in P. longimembris at about 18 days. This approximates the first change of growth constants (7.4%-2.3%), which occurs at 21 days.

Increases of other dimensions commonly used as indices of growth are given in Figs. 2-5. Growth in these structures, like total body weight, are multiphasic -- with total length, tail and hind foot all showing a four-part growth. Total length and tail length show their first growth inflections to lower values at about the 15th day and the second inflection at the 26-27th. These data agree with those presented for the kangaroo rat, D. merriami (Chew and Butterworth, 1963).

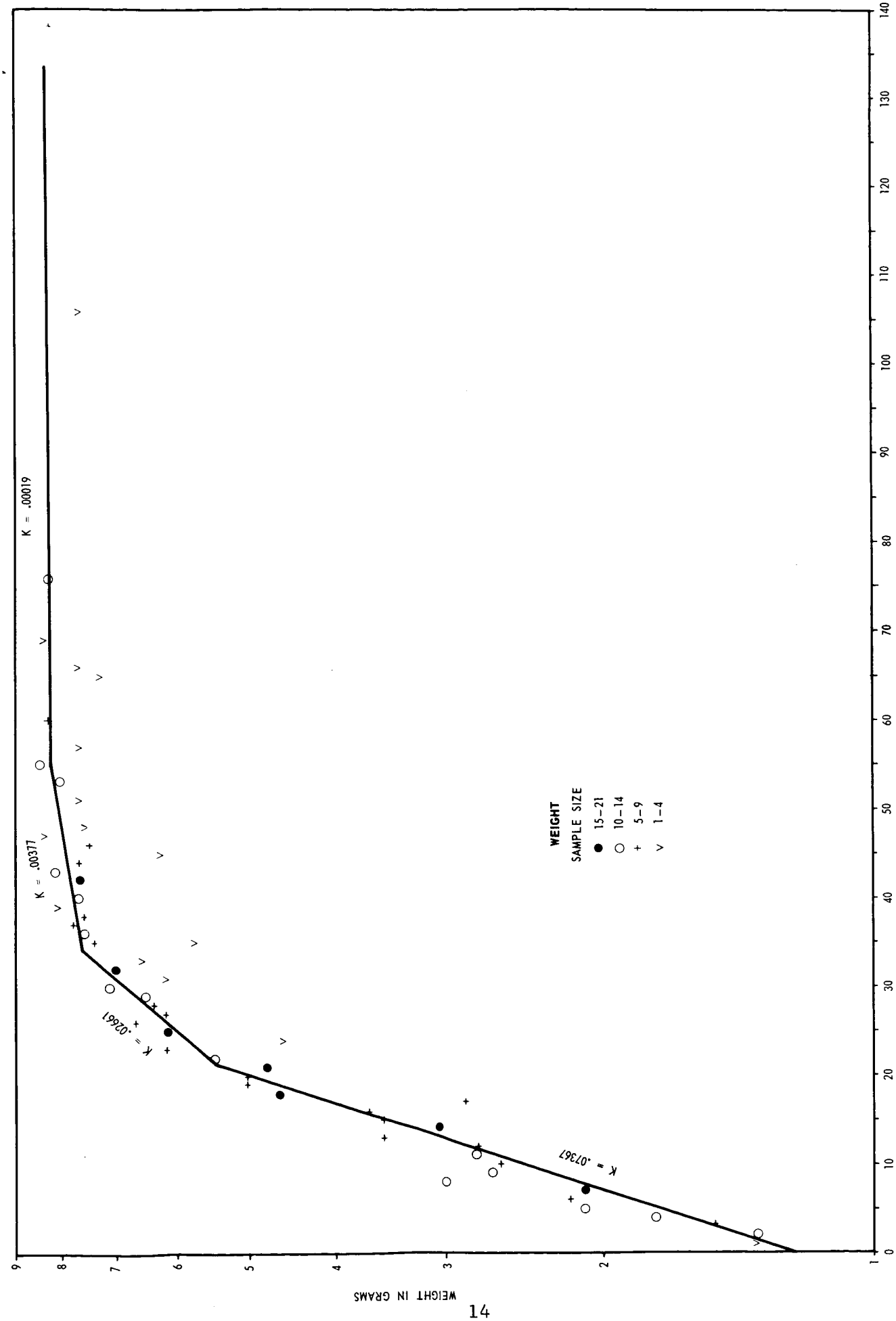

FIGURE 1 - Weight increase during growth of Perognathus longimembris; semilogarithmic plot

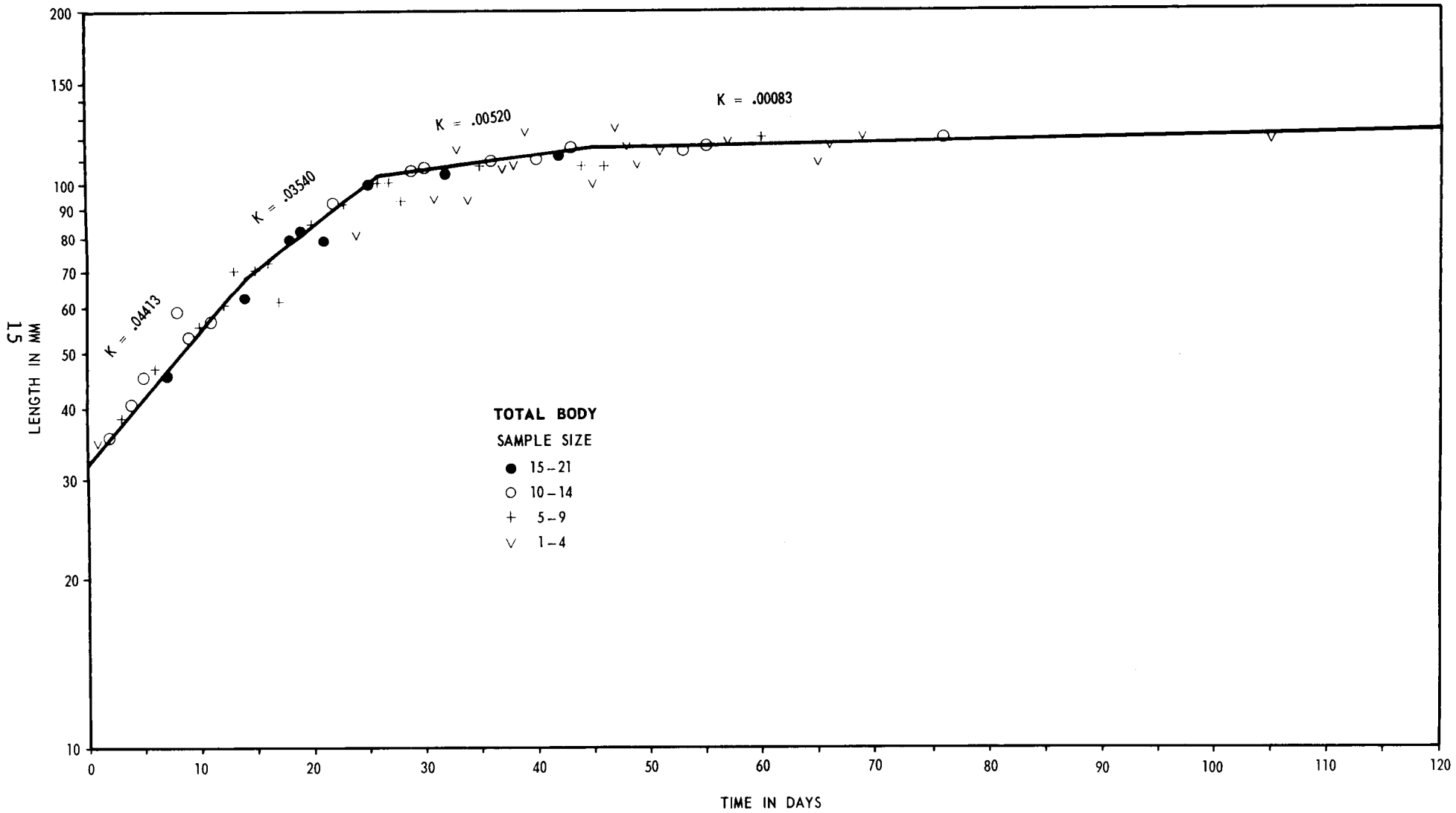

FIGURE 2 - Increase in total length in Perognathus longimembris; semilogarithmic plot

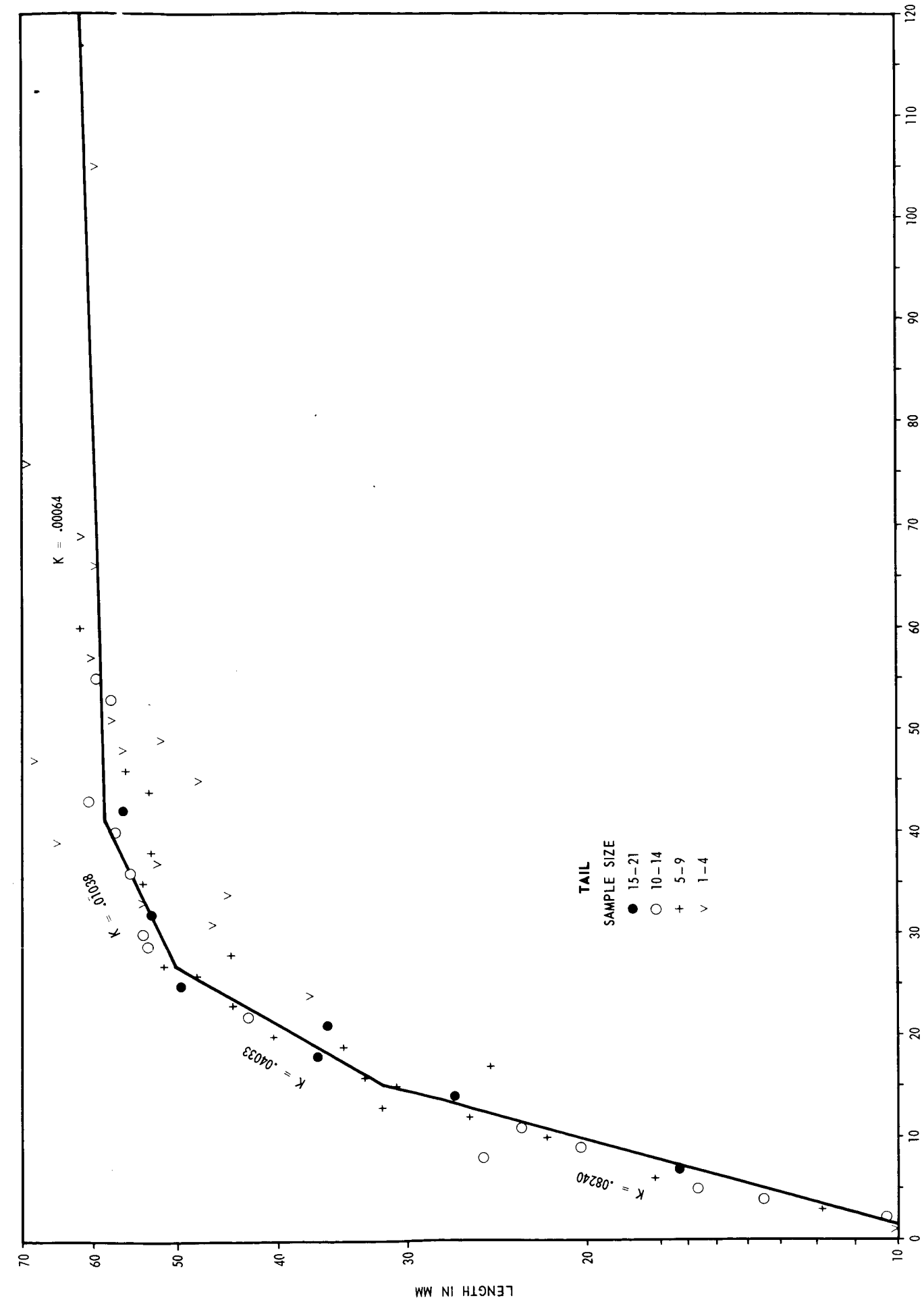

FIGURE 3 - Increase in tail length in Perognathus longimembris; semilogarithmic plot

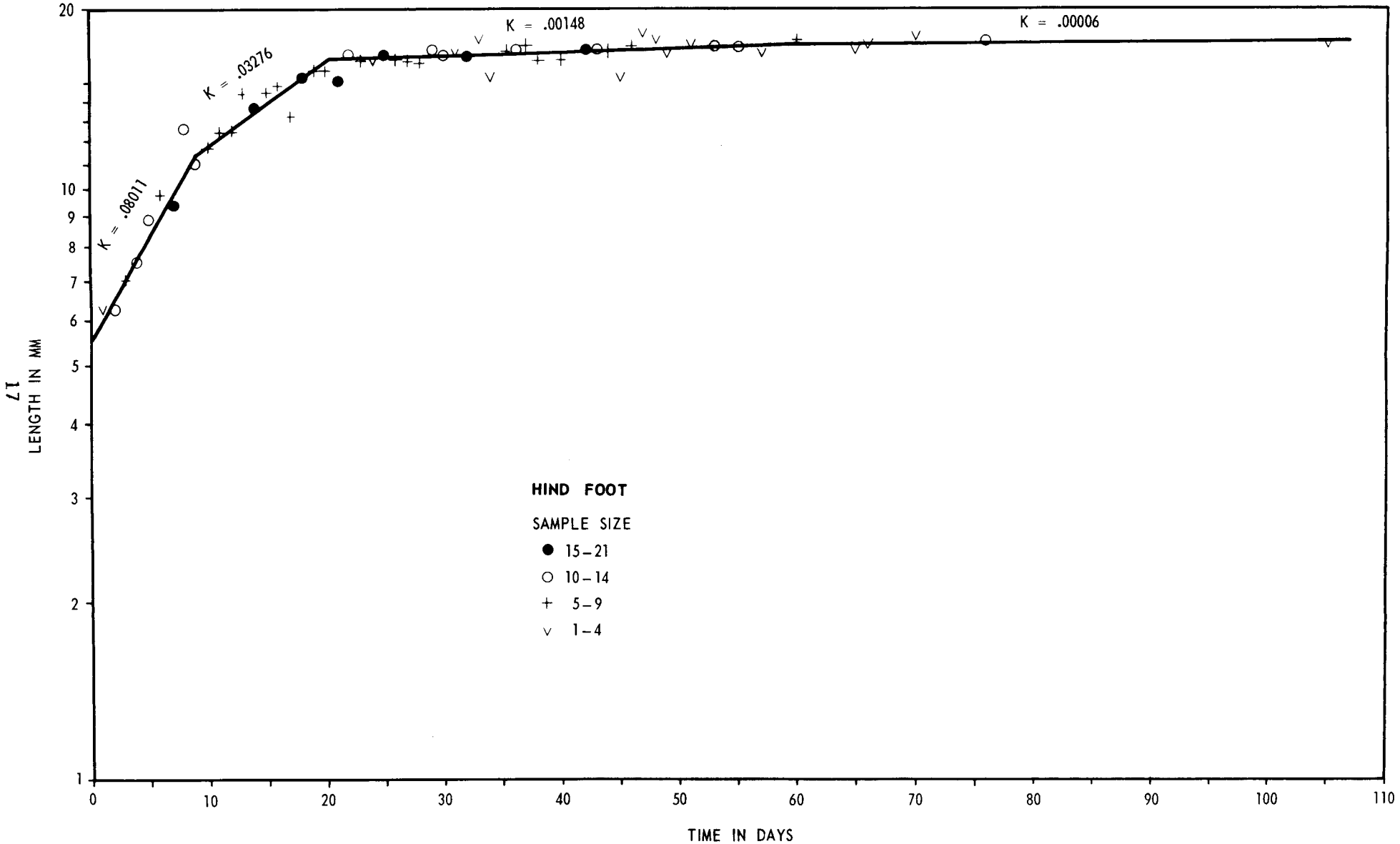

FIGURE 4 - Increase in hind foot length in Perognathus longimembris; semilogarithmic plot

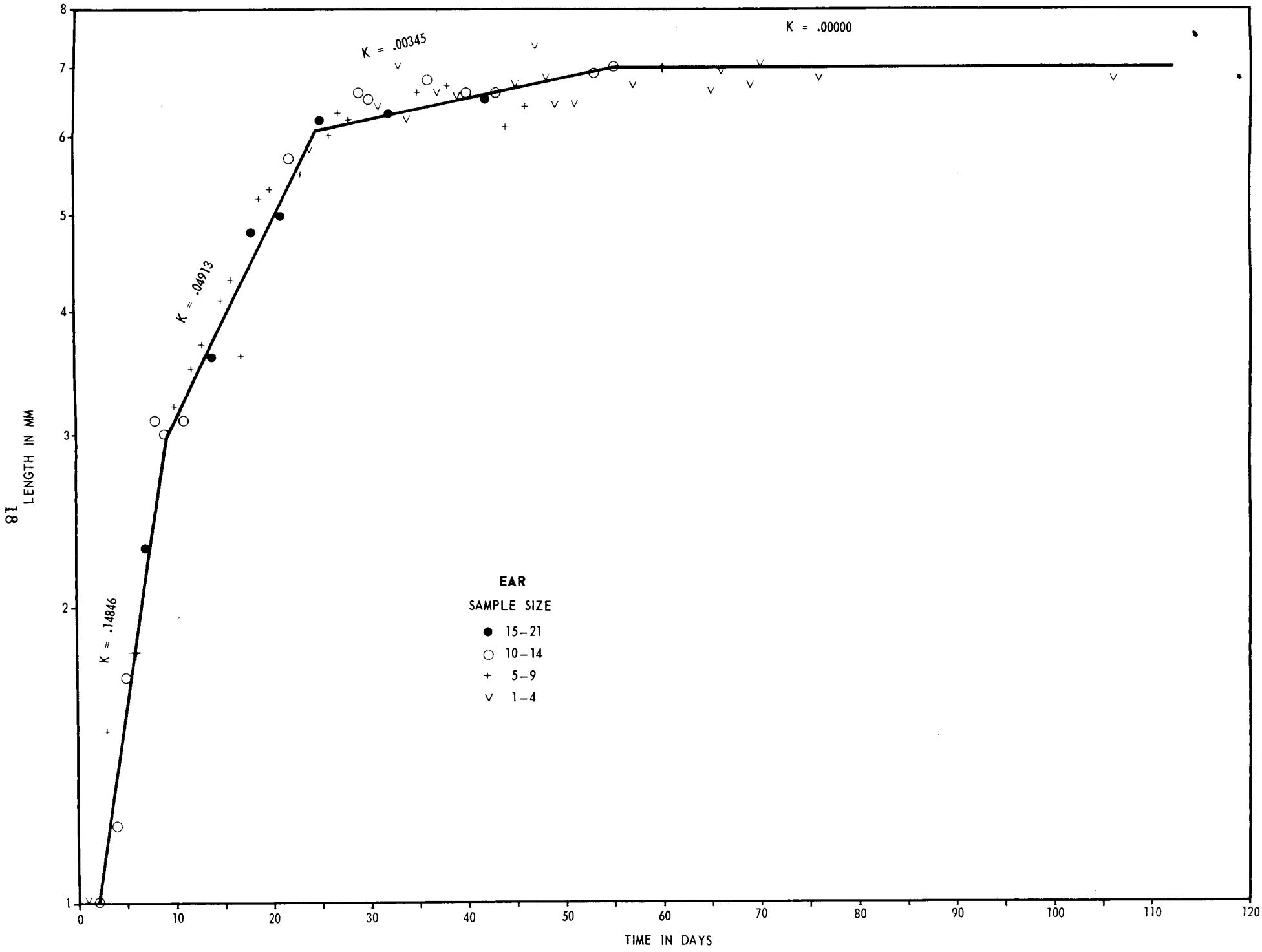

FIGURE 5 - Increase in ear length in Perognathus longimembris; semilogarithmic plot

The hind foot showed a four-part growth curve but was unique in that a near adult-size foot was attained at about 20 days. This means that when the animal attained about 70% of its total length and about 60% of its weight, it had an adult-size foot. Although this species is not as dependent upon saltatorial locomotion as the genus Dipodomys, this mode of travel is used during escape attempts and at other times when maximum speed is necessary. The adaptive value of a fast-developing foot is self-evident.

The ear showed the highest rate of growth, with a value of about 15% during the first 9 days. The inflections of growth rate are similar to those documented in the hind foot. Nearly 86% of adult size was reached by 25 days, with adult size being attained at about 55 days.

Summary and Conclusions

Data are presented on the growth and development of 26 individuals from 8 litters, 22 of which were the result of the first recorded matings of Perognathus longimembris in captivity. Newborn of this species of pocket mouse are naked and have a nearly transparent integument; pigmentation gradually fills the area above the future lateral line and is complete with sparse hair by day 9. Eyes and ear are sealed at birth with the pinnae developing at about day 4. The external meatus appears to open after day 13. Eyes usually opened between day 14-15. Cheek pouches were not present at birth, but started to develop on day 3 and were functional by day 14.

Semilogarithmic plots of body weight, total length, and lengths of tail, hind foot and ear showed polyphasic growth. All showed a four-part pattern with initial instantaneous percentage growth rates which varied from 4.4% for total length to 14.8% for ear. Hind foot showed the most rapid growth, reaching near adult size by 20 days.

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